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Electrophoretic protein patterns for the five strains of *Artemia* were compared in an effort to differentiate the strains. No two strains, when paired, showed identical banding patterns. Strains from San Pablo Bay and Utah had the most bands in common (81%) of any pair, and strains from Australia and Brazil had the fewest bands in common (30%). Whether this variation in electrophoretic protein patterns may occur among batches of one strain of *Artemia*, as well as among many strains, remains a question. Based on our preliminary study, there seems to be a need for future work in this area to test for the effect of temporal and environmental factors on the protein patterns of *Artemia*.

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International Study on *Artemia*[1] XI. Amino acid composition and electrophoretic protein patterns of *Artemia* from five geographical locations

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Abstract

The cysts of five geographical strains of *Artemia* (Australia, Brazil, Italy, Utah-USA, and San Pablo Bay, California-USA) were hatched and the newly hatched nauplii were acid hydrolyzed and analyzed for their amino acid content (Technicon Amino Acid Analyzer). Strains from Australia, Brazil, Italy, and Utah were similar in amino acid content, whereas the San Pablo Bay, California strain exhibited several values different from the other four strains. Essential amino acid levels for all five strains of *Artemia* were considered adequate in terms of the dietary requirement levels for Chinook salmon and levels that appear to be adequate for Atlantic silversides (Seidel *et al.*, 1980).

Electrophoretic protein patterns for the five strains of *Artemia* were compared in an effort to differentiate the strains. No two strains, when paired, showed identical banding patterns. Strains from San Pablo Bay and Utah had the most bands in common (81%) of any pair, and strains from Australia and Brazil had the fewest bands in common (30%). Whether this variation in electrophoretic protein patterns may occur among batches of one strain of *Artemia*, as well as among many strains, remains a question. Based on our preliminary study, there seems to be a need for future work in this area to test for the effect of temporal and environmental factors on the protein patterns of *Artemia*.

Introduction

Considerable dependence has been placed on brine shrimp (*Artemia*) as a food source in culturing fish and crustacean larvae. As a result, *Artemia* cysts have become in great demand, creating commercially high prices. Despite their relatively limited occurrence in nature, a number of geographical strains of brine shrimp are known and available.

In the past, success in fish culture operations has varied according to the specific strain of brine shrimp used. Whether this is due to toxic contamination (Bookhout and Costlow, 1970),

[1] International Interdisciplinary Study on *Artemia* Strains coordinated by the Artemia Reference Center, State University of Ghent, Belgium.

biochemical composition (Seidel *et al.*, 1980) or nutritional quality (Wickins, 1972), however, remains a question.

With a variety of brine shrimp strains now available, there is a need to provide aquaculturalists with reliable information in order that they may choose the most effective diet for a particular species. The diet's nutritional quality is an area of considerable importance. Dietary protein, for example, accounts for the maintenance of fish body tissues, the repletion of depleted tissues and the formation of new, additional protein (Cowey and Sargent, 1972). In view of its importance, this paper presents a biochemical analysis of amino acids and electrophoretic patterns of protein for five geographical strains of brine shrimp. Other biochemical characteristics of *Artemia* strains, such as lipids and caloric content (Schauer *et al.*, 1980), pigments (Soejima *et al.*, 1980) and pesticides and heavy metals (Olney *et al.*, 1980) will be covered separately.

Materials and methods

Sources and culturing of brine shrimp

Dehydrated cysts from five geographical locations were provided by the Artemia Reference Center (Ghent, Belgium). The origin of these cysts were Shark Bay, Australia (World Ocean, lot no. 113); Macau, Brazil (Companhia Industrial do Rio Grande do Norte, CIRNE-Brand, harvested 1978); Margherita di Savoia, Italy (harvested 1977); Great Salt Lake, Utah, USA (harvested 1977); San Pablo Bay, California, USA (Living World, San Francisco Bay Brand, Inc., lot no. 1628).

The cysts were rehydrated and hatched into nauplii according to procedures of Beck *et al.* (1980) at the Environmental Protection Agency (EPA), Environmental Research Laboratory (ERLN), Narragansett, Rhode Island, USA. Each strain was harvested as newly hatched nauplii, labeled and frozen at -20 °C until the time of analysis.

Protein hydrolysis and amino acid analysis

Frozen nauplii of each strain of brine shrimp were thawed to room temperature and were then acid hydrolyzed in duplicate. The procedure involved the *in vacuo* hydrolysis of protein material at 110 °C for 22 hr with 3.0 ml of 6.0 N HCl. Heavy walled, vacuum hydrolysis tubes were prepared for use by washing in a chromic acid cleaning solution, then in a mixture of H_2SO_4 and HNO_3 (3:1), rinsed overnight in deionized water and oven dried. The hydrolysate was filtered through a Buchner funnel (size 15-M) and rotary evaporated *in vacuo* at 37 °C. To insure removal of HCl, the residue was wet three times with 1 ml of deionized water and dried in the rotary evaporator. The free amino acid residue was then dissolved in 3-5 ml of pH 2.2 sodium citrate buffer, filtered through a 0.45 μ Millipore filter and stored under nitrogen at -20 °C until final dilution was made just prior to time of analysis.

Amino acid analyses were performed on a NC-2P Technicon Auto Analyzer equiped with a 25×0.5 cm cationic exchange column packed with Chromobead Resin, Type C-3 (ion exchange capacity of 5 meq/mg ; 8% cross-linked). The column was operated at 55 °C with a flow rate of 0.5 ml/min. A Columbia Scientific Instruments Supergrator-2 Integrator/Calculator was used to compute the absolute amount of each amino acid using color response

factors as determined from an authentic standard mixture of amino acids obtained from Pierce Chemical Co., Rockford, Illinois, USA.

Electrophoresis

Artemia nauplii from Australia, Brazil, Utah, Italy, and San Pablo Bay-California were run according to two electrophoretic techniques. The first was thin layer polyacrylamide gel isoelectric focusing (TLIEF), a technique developed by Kryznowek and Wiggin (1979). *Artemia* nauplii were focused in this manner at the National Marine Fisheries Service Gloucester Laboratory, Gloucester, Massachusetts, USA. Samples were applied to the gel in two forms : as a 'drip' and as the 'whole nauplii'. Preparation of the 'drip' was as follows : Each sample was thawed to room temperature, then approximately 2-4 g (wet wt) was centrifuged at 3 000 rpm for 30 min at -2 °C. The supernatant (liquid from thawing process) was spotted onto filter paper tabs on the gel in 10 and 20 μl amounts. The solid residue in the centrifuge tube (freshly prepared as above) was the 'whole nauplii' sample. It was spotted into gel wells twice for a total of 14 μl in the first 3-4 hr of the run in order to concentrate the protein. All runs were at 1 Watt constant power for 20 hr to a maximum of 500 volts. Gels were stained according to Righetti and Drysdale (1974).

The second electrophoretic technique tried was that of Saravis and Zamcheck (1979) and Saravis *et al.* (1979), and it involved rapid isoelectric focusing of proteins in a thin layer agarose gel. The thawed *Artemia* nauplii (5 μl) were applied directly to the gel in this method and were focued 10 min at 10 mA constant power, then 15 additional min at 25 W constant power. The five *Artemia* strains were focused in this manner at Boston City Hospital, Boston, Massachusetts, USA.

Results

Amino acids

The amino acid profiles for five strains of *Artemia* nauplii are presented in Table I. Values for essential and nonessential amino acids vary only slightly between strains from Australia, Brazil, Utah, and Italy. There are, however, several variations between the above four strains and the San Pablo Bay, California strain, including values for serine, glutamic acid, proline, phenylalanine, and lysine.

The essential amino acid requirements for Chinook salmon are presented in Table II. All five strains of brine shrimp appear to meet these requirement levels with the exception of phenylalanine in the Brazilian strain and valine in the Italian strain. These two exceptions, however, are only very slightly lower than the requirement levels.

Electrophoresis

Brine shrimp nauplii were run in a polyacrylamide gel (Kryznowek and Wiggin, 1979). Preliminary focusing was tried in the pH 5-7 range with San Pablo Bay *Artemia*, however bands were seemingly too numerous to be meaningful (Fig. 1). Fewer, and therefore more potentially unique bands were seen in basic pH ranges than in acidic pH ranges. The most useful banding patterns were seen in the range of pH 7-9 (Fig. 2).

No two strains of brine shrimp showed identical banding patterns in the pH 7-9 range when 'whole nauplii' samples were focused. The San Pablo Bay and Utah strains had 81% of their bands in common, followed by Italy-Brazil (74%), San Pablo Bay-Brazil (71%), Italy-San Pablo Bay (70%), Italy-Utah (64%) and Brazil-Utah (63%) (Fig. 3). No other combinations of the five strains showed over 50% common bands. Brine shrimp from Australia and Brazil had only 30% of their bands in common, which was the least of any two strains in the pH 7-9 range.

TABLE I

Amino acid profiles for *Artemia* nauplii from five geographical locations expressed as g amino acid/100 g protein (tryptophan destroyed by HCl)

Amino acid	Australia	Brazil	San Pablo Bay	Utah	Italy
Aspartic acid	10.8	11.0	14.1	11.3	11.2
Threonine	5.5	5.2	6.0	4.8	5.5
Serine	5.9	4.5	7.7	5.4	5.1
Glutamic acid	16.3	13.1	10.2	13.5	14.5
Proline	5.4	5.7	4.9	5.9	5.9
Glycine	5.7	6.0	7.4	6.0	7.2
Alanine	5.4	4.6	4.2	4.9	4.9
Valine	5.4	5.3	5.5	5.2	3.1
Methionine	2.8	2.2	2.6	3.7	3.7
Isoleucine	4.9	5.6	5.4	6.8	6.4
Leucine	7.9	8.9	8.4	10.0	10.1
Tyrosine	7.3	10.5	7.7	6.6	5.4
Phenylalanine	7.7	5.1	10.4	8.5	8.5
Histidine	3.8	4.9	3.5	2.7	3.8
Lysine	10.6	11.7	8.7	9.3	10.7
Arginine	10.9	11.5	9.8	9.7	9.8

TABLE II

Essential amino acids for *Artemia* nauplii from five geographical locations and Chinook salmon dietary requirements for these amino acids (expressed as g amino acid/100 g protein)

Amino acid	Chinook salmon	Australia	Brazil	San Pablo Bay	Utah	Italy
Threonine	2.5	5.5	5.2	6.0	4.8	5.5
Valine	3.25	5.4	5.3	5.5	5.2	3.1
Methionine	1.5	2.8	2.2	2.6	3.7	3.7
Isoleucine	2.5	4.9	5.6	5.4	6.8	6.4
Leucine	4.0	7.9	8.9	8.4	10.0	10.1
Phenylalanine	5.25	7.7	5.1	10.4	8.5	8.5
Histidine	1.75	3.8	4.9	3.5	2.7	3.8
Lysine	5.0	10.6	11.7	8.7	9.3	10.7
Arginine	5.75	10.9	11.5	9.8	9.7	9.8

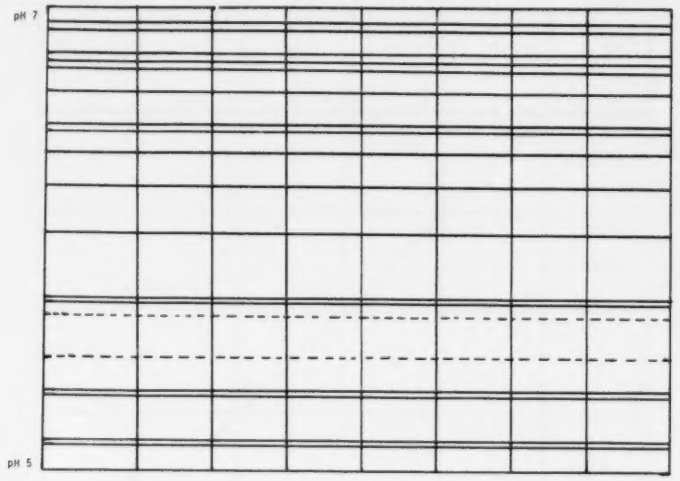

FIG. 1. Electrophoretic banding patterns of San Pablo Bay *Artemia* nauplii in polyacrylamide gel at pH 5-7 (8 replicates). Dotted lines indicate lightly stained protein bands. (Sketched from photograph of gel).

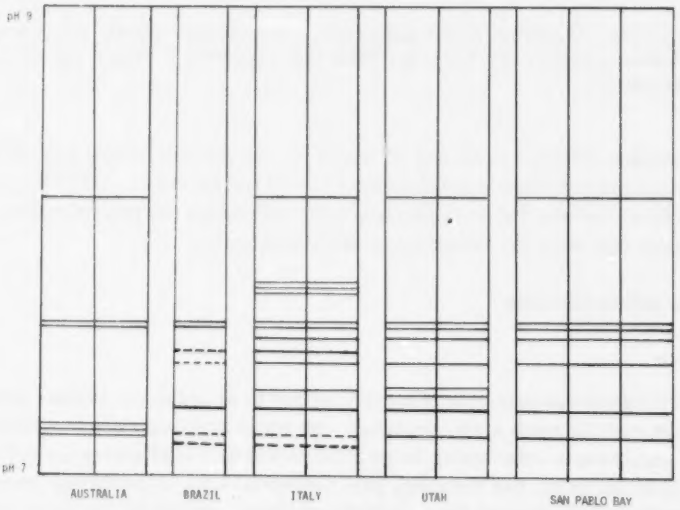

FIG. 2. Electrophoretic banding patterns of *Artemia* nauplii from five geographic locations in polyacrylamide gel at pH 7-9 : Australia, 2 replicates ; Brazil, 1 replicate ; Italy, 2 replicates ; Utah, 2 replicates ; San Pablo Bay, 3 replicates. Dottes lines indicate lightly stained protein bands. (Sketched from photograph of gel).

COMMON / DIFFERENT	AUSTRALIA	BRAZIL	ITALY	UTAH	S.P. BAY, CALIF.
AUSTRALIA		15	16	20	23
BRAZIL	69		35	31	36
ITALY	68	30		32	35
UTAH	60	31	36		44
S.P. BAY, CALIF.	54	29	30	13	

FIG. 3. Common and different protein bands between *Artemia* "strain-pairs" from five geographical locations : Australia, Brazil, Italy, Utah, San Pablo Bay, California. (Amounts expressed as % total bands/"strain-pair").

A second electrophoretic technique, in which the five *Artemia* strains were focused in an agarose gel according to Saravis and Zamcheck (1979) and Saravis *et al.* (1979), provided very rapid resolution of bands. The wide pH range (pH 3-10) used in this clinical method, however, showed bands that were too numerous to easily differentiate.

Discussion and conclusions

AMINO ACIDS

In order for dietary protein to be effectively utilized by an organism, its essential amino acid pattern must meet the needs of that organism. The amino acid composition of brine shrimp is, therefore, important in determining its potential worth as a food source for culturing fish.

Amino acid values for San Francisco Bay, California, USA brine shrimp have previously been reported by Gallagher and Brown (1975) and Claus *et al.* (1979). Values for brine shrimp from Great Salt Lake, Utah have also been reported by Claus *et al.* (1979). In comparing the above amino acid profiles with those of the current study for San Pablo Bay, California and Utah strains, differences may be found in values for glycine, alanine, valine, methionine, tyrosine and phenylalanine. These differences may be due to variations in the age of *Artemia*

analyzed, variations in year batches and/or year locations of *Artemia*, or differences in methods of analysis.

In an earlier study (Seidel *et al.*, 1980), a variety of live and artificial diets, including 3-day-old brine shrimp, were fed to Atlantic silverside (*Menidia menidia*) larvae. Biochemical analyses were performed on all diets and fish in each diet group. There was very little variation found in the amino acid composition of test organisms regardless of diet treatment, implying that once minimum requirement levels of essential and nonessential amino acids were met, any additional dietary protein was utilized as energy or was otherwise eliminated.

In the current study, nauplii from five strains of brine shrimp adequately meet the minimum essential amino acid requirements of Chinook salmon (Mertz, 1969). This implies that in terms of protein quality, any of the five brine shrimp strains could support a fish such as Chinook salmon equally well. Therefore, once the minimum requirements for essential amino acids of a fish species is known, an adequate dietary protein source may be selected.

Electrophoresis

Thin layer isoelectric focusing has been employed successfully in the differentiation of uncooked fish species (Lundstrom, 1979) and cooked crab species (Kryznowek and Wiggin, 1979). In our study, *Artemia* were similarly focused in an attempt to differentiate strains on the basis of protein patterns.

Protein banding patterns in the pH 5-7 range were observed in preliminary focusing of San Pablo Bay *Artemia* (Fig. 1). The great number of bands in this range led to focusing in the pH 7-9 range since it seemed very unlikely to be able to see unique bands in the acidic range.

When 'whole *Artemia* nauplii' samples were focused from pH 7 (anode at bottom) to pH 9 (cathode at top) (Fig. 2), banding patterns clearly showed that no two strains of *Artemia* were identical in protein composition. Banding patterns of every combination of two strains of brine shrimp (of the five strains studied) were compared. Bands held in common between each strain-pair, as well as those which differed, were counted and recorded as a percentage of the total number of bands in the given pair (Fig. 3). Common protein bands between members of a pair were positively correlated with strain relationship, whereas different bands were negatively correlated. Of the ten pair combinations, only the San Pablo Bay and Utah strains showed greater than 80% common bands ; five strain-pairs showed 60-80% common bands and four strain-pairs showed fewer than 50% common bands (Fig. 3).

In terms of pairs with the greatest percentage of common bands, and in descending order, a tentative listing of relationship between pairs of five strains of *Artemia* may be formed : 1) San Pablo-Utah, 2) Italy-Brazil, 3) San Pablo-Brazil, 4) Italy-San Pablo, 5) Italy-Utah, 6) Brazil-Utah, 7) Australia-San Pablo, 8) Australia-Utah, 9) Australia-Italy, 10) Australia-Brazil. This data suggests that the San Pablo, Italy, Utah, and Brazil strains are at least moderately related, although none are identical. These four strains of *Artemia*, however, appear to be different from the Australian strain.

In this preliminary attempt to electrophoretically separate whole body proteins from five strains of *Artemia*, several objectives were achieved. The basic pH range (7-9) showed more potentially unique bands than acidic pH ranges, a 'whole nauplii' sample applied directly into the gel gave better protein concentrations and resolution of bands than a 'drip' sample, and a urea-based gel was more effective than an agarose gel. Future work with *Artemia* electrophoresis may further refine the above objectives as well as advancing the technique in other

ways. The present findings, however, seem to provide useful 'fingerprinting' data which could be used in the quick identification of unknown *Artemia* strains. It is possible, however, that electrophoretic data would show as much variation among several batches of one *Artemia* strain as it does here among several strains. If this is the case, its reliability as a quick identification method may be limited.

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Literature cited

Beck A. D., D. A. Bengtson, and W. H. Howell. 1980. International Study on *Artemia*. V. Nutritional value of five geographical strains of *Artemia* : effects on survival and growth of larval Atlantic silverside *Menidia menidia*. p. 249-259. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Bookhout C. G. and J. D. Costlow, Jr. 1970. Nutritional effects of *Artemia* from different locations on larval development of crabs. *Helgoländer wiss. Meersunters.* 20:435-442.

Claus C., F. Benijts, G. Vandeputte, and W. Gardner. 1979. The biochemical composition of the larvae of two strains of *Artemia salina* (L.) reared on two different algal foods. *J. exp. mar. Biol. Ecol.* 36:171-183.

Cowey C. B. and J. R. Sargent. 1972. Fish nutrition. *Adv. Mar. Biol.* 10:383-492.

Gallagher M. and W. D. Brown. 1975. Composition of San Francisco Bay brine shrimp (*Artemia salina*). *Agr. and Food Chem.* 23(4):630-632.

Kryznowek J. and K. Wiggin. 1979. Identification of species in cooked crabmeat by thin layer isoelectric focusing. *J. Assoc. Off. Anal. Chem.* 62(3):630-636.

Lundström R. C. 1979. Fish species identification by thin layer isoelectric focusing. *J. Assoc. Off. Anal. Chem.* 62(3):624-629.

Mertz E. T. 1969. Amino acid and protein requirements of fish. p. 233-244. *In* : Neuhaus O. W. and J. E. Halver (Eds). Academic Press, New York. 311 p.

Olney C. E., P. S. Schauer, S. McLean, You Lu, and K. L. Simpson. 1980. International Study on *Artemia*. VIII. Comparison of the chlorinated hydrocarbons and heavy metals in five different strains of newly hatched *Artemia* and a laboratory-reared marine fish. p. 343-352. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Righetti P. H. and J. W. Drysdale. 1974. Isoelectric focusing in gels. *J. Chromatogr.* 98:271-321.

Saravis C. A. and N. Zamcheck. 1979. Isoelectric focusing in agarose. *J. Immunological Methods* 29:91-96.

Saravis C. A., M. O'Brien and N. Zamcheck 1979. Direct tissue isoelectric focusing in agarose. *J. Immunological Methods* 29:97-100.

Schauer P. S., D. M. Johns, C. E. Olney, and K. L. Simpson. 1980. International Study on *Artemia*. IX. Lipid level, energy content and fatty acid composition of the cysts and newly hatched nauplii from five geographical strains of *Artemia*. p. 365-373. *In* : The brine shrimp *Artemia*. Vol. 3. Ecology, Culturing, Use in Aquaculture. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 456 p.

Seidel C. R., P. S. Schauer, T. Katayama, and K. L. Simpson. 1980. Culture of Atlantic silversides fed on artificial diets and brine shrimp nauplii. *Bull. Jap. Soc. Sci. Fish.* 46(2):237-245.

Soejima T., T. Katayama, and K. L. Simpson. 1980. International Study on *Artemia*. XII. The carotenoid composition of eight geographical strains of *Artemia* and the effect of diet on the carotenoid composition of *Artemia*. p. 613-622. *In* : The brine shrimp *Artemia*. Vol. 2. Physiology, Biochemistry, Molecular Biology. Persoone G., P. Sorgeloos, O. Roels, and E. Jaspers (Eds). Universa Press, Wetteren, Belgium. 664 p.

Wickins J. F. 1972. The food value of brine shrimp, *Artemia salina* L., to larvae of the prawn, *Palaemon serratus* Pennant. *J. exp. mar. Biol. Ecol.* 10:151-170.